Mixture effects of twenty-seven environmental contaminants given to rats at doses comparable to human exposure

Hadrup, Niels; Svingen, Terje; Egebjerg, Karen Mandrup; Pedersen, M.; Frederiksen, H.; Skov, Kasper; Frandsen, Henrik Lauritz; Vinggaard, Anne Marie

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

DTU Food
National Food Institute

Mixture effects of 27 environmental contaminants given to juvenile male rats at doses comparable to human exposure

N Hadrup[1], T Svingen[1], K Mandrup[1], M Pedersen[2], H Frederiksen[3], K Skov[2], HL Frandsen[2] and AM Vinggaard[1]

[1]Division of Toxicology and Risk Assessment, [2]Division of Food Chemistry, National Food Institute, Technical University of Denmark, Søborg, Denmark.
[3]Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark.

Summary

Safe levels of exposure are typically estimated from single compounds without consideration of potential combination effects of several chemicals present at low levels. Here we have assessed various biological endpoints in rats having been exposed to a mixture of 27 environmental chemicals during juvenile age at levels comparable to human exposure. We found exposed rats to have compromised growth rates. Exposed animals showed no significant changes in steroid hormones, but displayed hepatotoxicity already at low level exposure. Metabolic homeostasis was also affected. We conclude that a realistic mixture of 27 chemicals, designed from knowledge on human internal exposure levels, can leave adverse footprints in young male rats.

Table: Low mix levels listed (Mid = x3; High = x10)

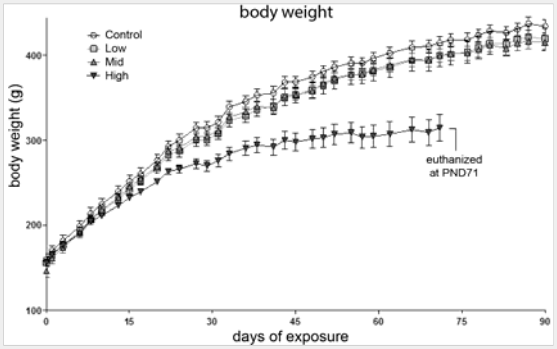
Compound	µg/kg bw/day	Compound	µg/kg bw/day
Acrylamide	4.000	cobalt(II)chloride	0.110
Benzophenon-3	2.600	leadchloride	21.500
Bisphenol A	10.000	mercury(II)chloride	4.700
Trichlosan	5.000	thallium(I)chloride	0.350
o-phenylphenol	0.060	PFOS	0.900
trans-nonachlor	0.350	PFNA	0.200
p,p-DDE/4,4'-DDE	13.000	mono-Butyl phthalate	62.000
2,4,6-trichlorophenol	10.000	AHTN	6.200
Chlorpyrifos	0.400	PCB 153	20.000
3-phenoxybenzoic acid	0.010	TCDD	0.034
Arsentrioxid	3.100	Benz[a]pyrene (PAHs)	0.400
bariumchloride,2H2O	0.230	PHIP	0.100
cadmiumchloride	0.200	MeIQx	0.050
cesiumchloride	0.610	**TOTAL**	**166.104**

LC-MS/MS analysis of several of the mixture chemicals in rat serum and urine confirmed absorption of the chemicals.

Results

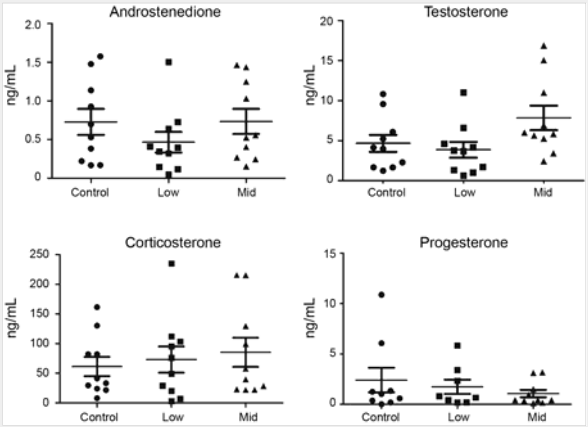
1. Mixture exposure affects growth rates

At start of exposure (6 week), all animals were comparable in size and weight. Exposed animals showed a stunted growth pattern over the 90 days, with severe effects on High-dose group already from 14 days.



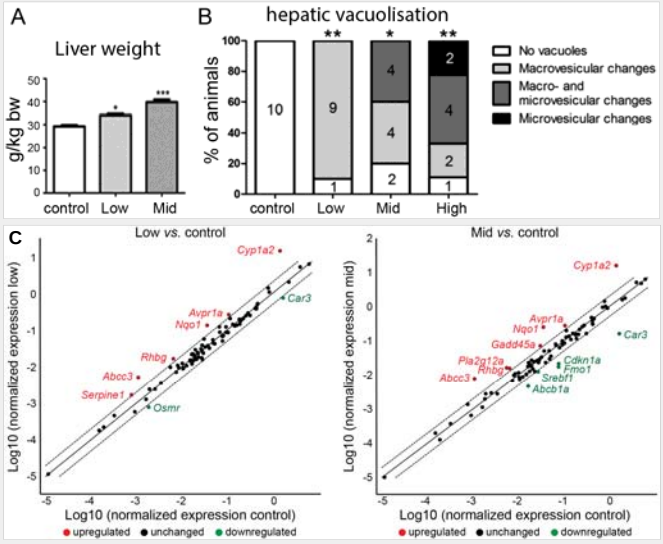
2. Mixture exposure do not adversely affect circulating steroid hormone levels

Blood serum steroid hormone levels were analysed by LC-MS/MS. No significant changes were observed between Low- and Mid-dose groups and control animals.



3. Mixture exposure causes hepatotoxicity at all doses

Following prolonged exposure, **A)** livers were significantly enlarged in the Low-dose group, with further increase in Mid-dosed animals. **B)** Hepatic vacuolisation was evident in most animals from exposed groups, with more severe pathologies in Mid- and High-dosed animals. **C)** Gene expression profiling (RT^2 Profiler^{TM} PCR array) confirmed liver toxicity, with changes to transcript levels of markers for e.g. cholestasis (*Abcb1a, Abcc3*) and hepatotoxicity (*Cyp1a2, Car3, Fmo1*).



4. Mixture exposure disrupts metabolic/lipid homeostasis

PLS DA plots of the blood metabolome show a clear separation (HPLC-MS) of phospholipids and lipids in exposed animals relative to control. There was also a clear dose-dependent effect with a greater separation between the Mid-dose and control groups, than between Low-dose and control groups. Analyses were performed on plasma collected at 30, 60 and 90 days after initiation of dosing regimen.